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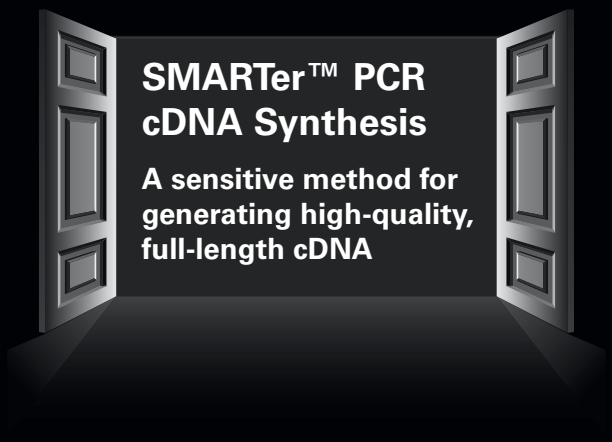
Volume XXIV, No. 2, 2009

CLONTECHniques

introducing



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Clontech

Coexpress MicroRNAs with Fluorescent Proteins

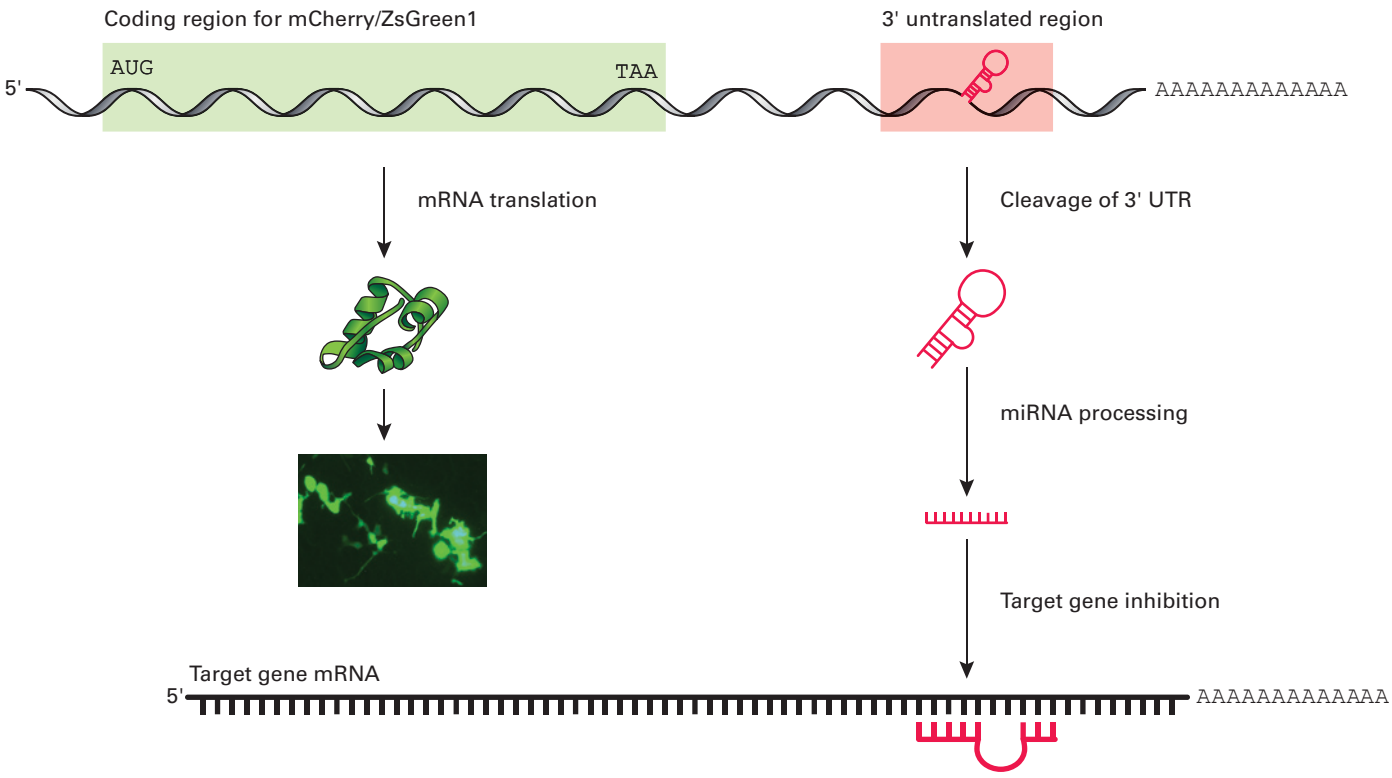
These vectors coexpress your miRNA with either the mCherry or ZsGreen1 fluorescent protein marker

- **High-level miRNA and fluorescent protein coexpression**
- **Simultaneously verify miRNA expression and transfection efficiency**
- **Very bright fluorescent proteins**

Clontech offers two new vectors that provide high-level miRNA expression and allow you to verify it with fluorescent protein expression. The **pmR-mCherry** and **pmR-ZsGreen1 Vectors** couple your miRNA expression cassette to a bright red or green fluorescent reporter, for miRNA expression you can see and select.

How Does It Work?

Clone your miRNA sequence into the vector's multiple cloning site (MCS) located in the 3' untranslated region of the fluorescent protein's mRNA transcript. This enables both molecules to be expressed simultaneously from the vector's strong CMV promoter. Each vector is equipped with the high-level CMV promoter, a selectable marker, and a fluorescent protein-miRNA expression cassette containing either mCherry or ZsGreen1—our two brightest Living Colors® Fluorescent Proteins (Figure 1). In short, you can clone and express your favorite miRNA, and then select, sort and/or visualize the cells in which it is expressed.



The pmR-mCherry and pmR-ZsGreen1 vectors will coexpress a fluorescent protein and an miRNA sequence that is embedded in the 3' UTR of the vector's mRNA transcript. miRNA expression can be selected for and/or verified in transfected cells by monitoring red or green fluorescence.

miRNA Expression

Once an miRNA sequence is cloned in the multiple cloning site of these vectors, miRNA expression can be delivered into any transfectable cell line. We used the pmR-ZsGreen1 and pmR-mCherry vectors to express several different miRNAs in 293T cells (Figure 2). The miRNA sequences were amplified from human genomic DNA and then cloned into the vectors. The sequences included the indicated miRNA stem-loop along with ~300 bp of flanking DNA. Following transfection into separate cultures, samples of total RNA were prepared and treated with DNase prior to quantifying the expressed miRNAs using Clontech's **Mir-X™ miRNA qRT-PCR SYBR® Kit.** Both vectors produced similarly high levels of expression for each miRNA, which were elevated to a range of values between 75- and >3000-fold over the vector-only controls.

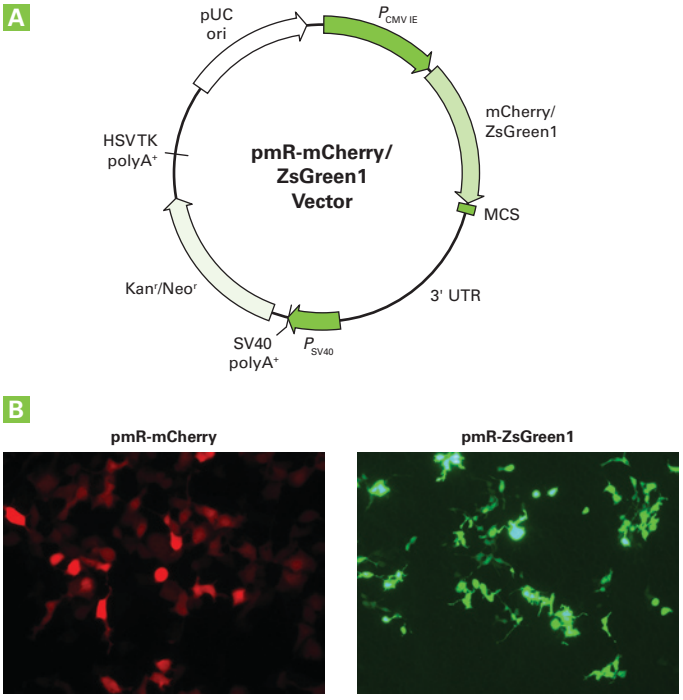


Figure 1. The pmR-mCherry and pmR-ZsGreen1 Vectors. Map of the vectors (**Panel A**). Cells transfected with the vectors exhibit red or green fluorescence (**Panel B**).

miRNA Quantification

Clontech's Mir-X miRNA qRT-PCR SYBR Kit has a diverse variety of applications, as it is able to detect and quantify multiple miRNAs, shRNAs, or mRNA targets in a single RNA sample. The complete, dual-function kit includes a fast and simple, one-step protocol for first-strand cDNA synthesis, as well as the reagents needed for the qPCR of your RNA target using SYBR Advantage® technology.

From verifiable expression to accurate miRNA analysis, Clontech has the highly effective, state-of-the-art tools you need for investigating any miRNA network.

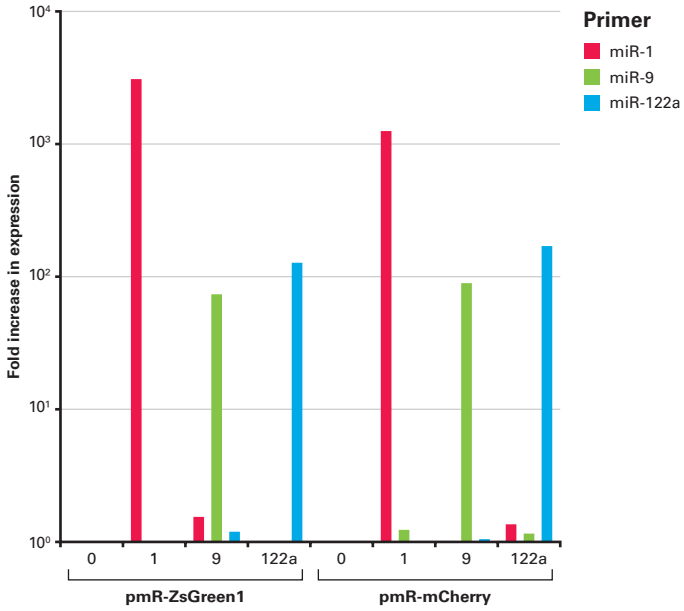


Figure 2. miRNA expression from pmR vectors. DNA sequences for the miR-1, miR-9, and miR-122 miRNAs were cloned into the pmR-ZsGreen1 and pmR-mCherry vectors, and the recombinant plasmids (1, 9, & 122a, respectively), as well as the parental vectors (0), were each transfected into separate cultures of 293T cells. After 48 hr, cells were harvested and the RNA was isolated for Mir-X miRNA qRT-PCR analysis using specific primers and the U6 snRNA as a normalization standard. Each primer was used with each RNA sample, but detected only the corresponding miRNA cognate.

Ordering Information			
Product	**Size**	**Cat. No.**	
pmR-ZsGreen1 Vector	20 µg	632541	NEW!
pmR-mCherry Vector	20 µg	632542	NEW!
Mir-X miRNA qRT-PCR SYBR Kit*	200 rxns 600 rxns	638314 638316	NEW!

* Includes a Mir-X miRNA First-Strand Synthesis Kit.

Visit our website for more details! click here...

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Mir-X™ MicroRNA Quantification

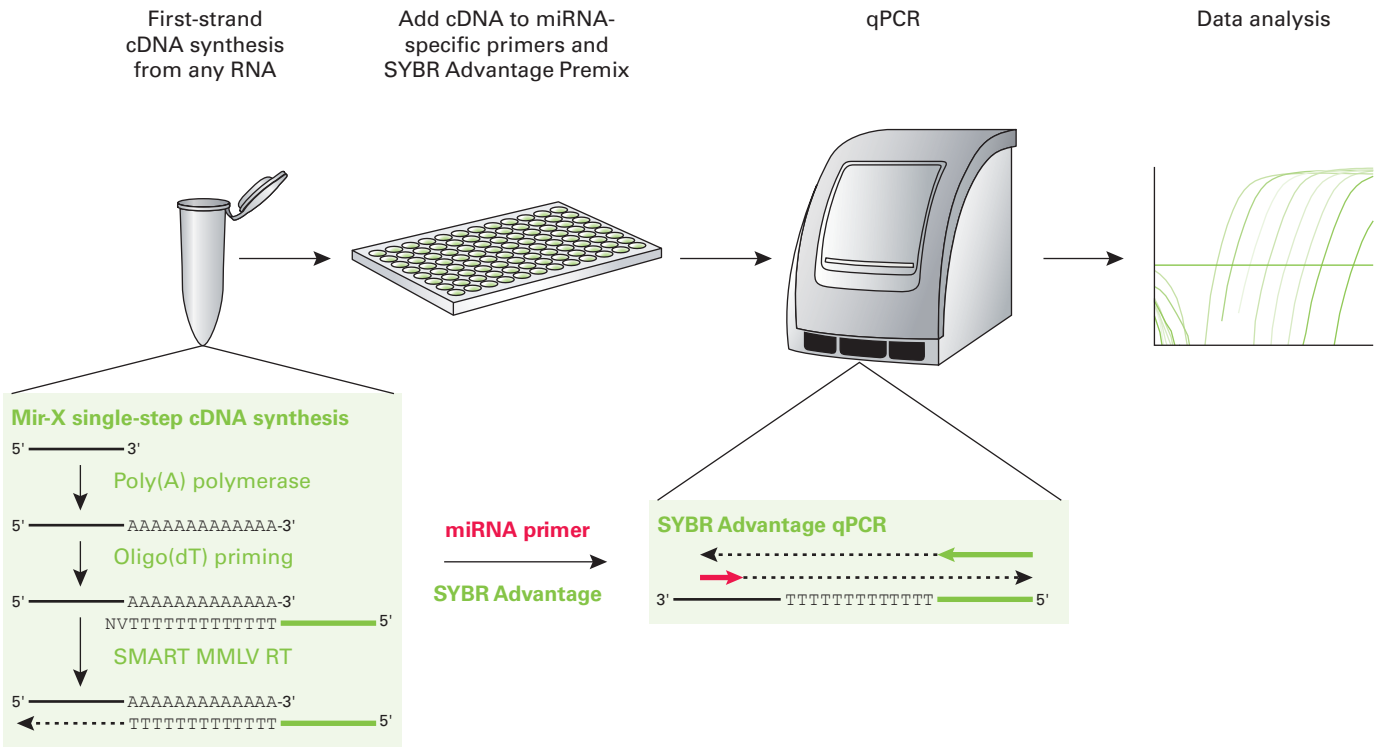
Quickly and accurately quantify your miRNAs and their mRNA targets with this complete qRT-PCR kit.

- **Quantify any miRNA and its target from the same RNA sample**
- **2-kits-in-1: cDNA synthesis and qPCR**
- **Simple, single-step cDNA synthesis reaction**

For the challenging task of unraveling microRNA (miRNA) expression profiles in tissues and cell lines, Clontech scientists have developed a sensitive, reliable, and easy-to-use miRNA quantification system. The **Mir-X miRNA qRT-PCR SYBR® Kits** are complete, dual-function systems for performing first-strand cDNA synthesis and quantitative PCR (qPCR) to precisely measure the level of your favorite miRNAs. The kits are available in economical, large-sized formats that provide 200 or 600 qPCR reactions.

Simple and Sensitive

A simple, single-step reaction uses an optimized mix of poly(A) polymerase and **SMART™ MMLV Reverse Transcriptase** to synthesize first-strand cDNA from your RNA sample. The cDNA is then specifically amplified and quantified by qPCR using your miRNA-specific primer and our **SYBR Advantage® qPCR Premix.** Multiple miRNA species, as well as the mRNA targets of the miRNAs, can be amplified from a single cDNA sample. The system is extremely sensitive and able to detect miRNAs down to 50 copies.



Mir-X miRNA qRT-PCR SYBR Kits use a single-step, single-tube reaction to produce first-strand cDNA, which is then specifically and quantitatively amplified using a miRNA-specific primer and SYBR Advantage qPCR chemistry. In the Mir-X cDNA synthesis reaction, RNAs are poly(A)-tailed using poly(A) polymerase, and then copied using a modified oligo(dT) primer and SMART MMLV Reverse Transcriptase.

Highly Specific Detection

To demonstrate the specificity of Mir-X miRNA quantification, we used a series of 8 highly similar synthetic Let7 miRNA variants (Figure 1). We first spiked each of the Let7 miRNAs into separate samples of yeast polyA+ RNA and generated cDNA using the Mir-X single-tube reaction. We then tested a panel of variant-specific primers with each cDNA sample to determine each primer's ability to specifically and individually quantify the Let7 subtypes in the cDNA sample. Despite the high degrees of similarity among the variants and the primers (Figure 1, Panel A), Mir-X qPCR was highly specific in detecting each Let7 variant (Figure 1, Panel B).

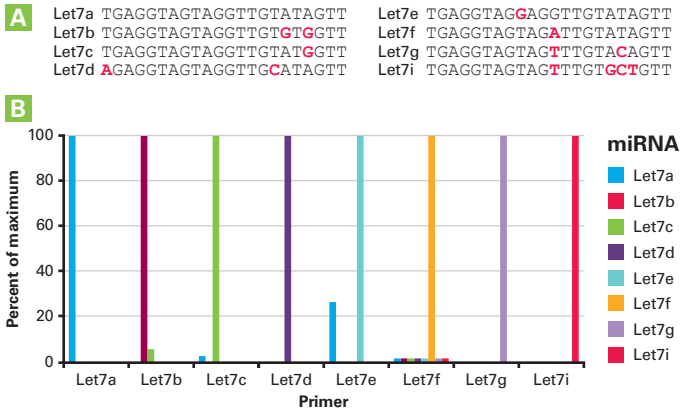


Figure 1. Specific quantification of Let7 miRNA variants. Using miRNA-specific primers (**Panel A**), Mir-X qRT-PCR was able to specifically detect and quantify each member of a series of 8 synthetic Let7 variants that had been spiked into a background of yeast polyA+ RNA (**Panel B**). The primers detected each of their corresponding Let7 miRNA cognates, but did not detect the off-target variants in 63 of 64 possible combinations.

Diverse Research Applications

Since the Mir-X system is able to detect multiple miRNAs, shRNAs, or mRNA targets in a single RNA sample, it can be used for a variety of applications. In principle, any RNA that is, or can be, polyadenylated may be quantified using the Mir-X method.
In mouse embryonic stem cells, we were able to monitor the alterations in expression for a panel of 12 miRNAs that respond to trichostatin A (TSA) treatment (Figure 2).

Exposing aggregated mouse P19 cell clusters to retinoic acid (RA) causes them to acquire neural cell phenotypes, which are accompanied by changes in the cellular miRNA pool. Using the Mir-X system, we tracked the abundance of one such miRNA, miR-9, which was induced by RA and continued to accumulate in these cells following a 5 day exposure to RA (Figure 3).

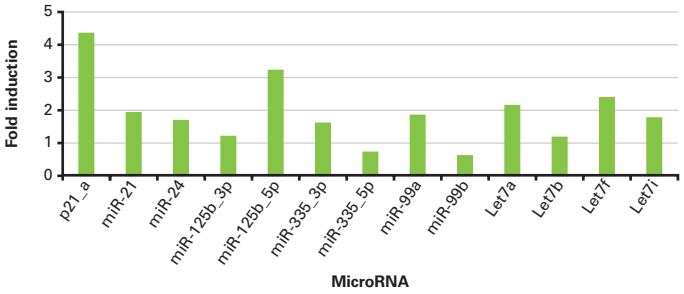


Figure 2. Trichostatin A treatment alters miRNA expression in mouse ES cells. Mouse embryonic stem cells were harvested either prior to or after being treated with trichostatin A (TSA) for 18 hr. RNA was prepared from the cells, and was then analyzed by Mir-X miRNA qRT-PCR using primers specific for the 12 indicated miRNAs and for a p21 control mRNA known to be induced by TSA.

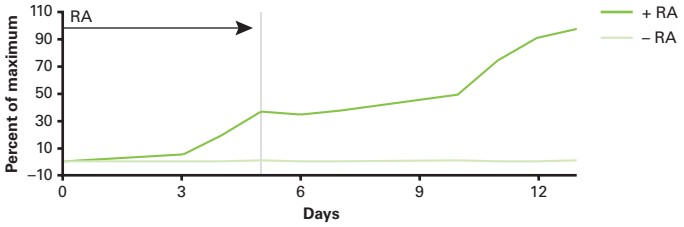


Figure 3. Induction of miR-9 in mouse P19 cells. P19 mouse embryonal carcinoma cells were plated on agarose-coated petri dishes and allowed to form embryoid bodies (EB) in the presence or absence of retinoic acid (RA). After five days of culture, EBs were dissociated with trypsin and replated on tissue culture dishes without RA. Cells were harvested on days 3–7 and 10–13, and the induction of miR-9 was followed and quantified using the Mir-X miRNA qRT-PCR SYBR Kit and primers specific for miR-9 and U6 (as a normalization control).

Summary

Mir-X miRNA qRT-PCR SYBR Kits are complete, dual-function qPCR systems that have the flexibility to monitor the level of your favorite miRNA or any other RNA species in your RNA sample. The single-tube cDNA synthesis is faster and far less complicated than other available methods, while the miRNA qPCR is very sensitive and extremely accurate.

Ordering Information

Product	Size	Cat. No.	
Mir-X miRNA qRT-PCR SYBR Kit*	200 rxns 600 rxns	638314 638316	NEW!
Mir-X miRNA First-Strand Synthesis Kit	20 rxns 60 rxns	638313 638315	NEW!

* Includes a Mir-X miRNA First-Strand Synthesis Kit.

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Synthesize cDNA the SMARTer™ Way

Get better results with SMARTer PCR cDNA Synthesis Kits

- **Generate high-quality cDNA from as little as 1–2 ng of total RNA**
- **Preserve precious samples and maintain accurate gene representation**
- **Higher specificity, lower background, and increased yield**
- **Enrich for full-length cDNA**
- **No adaptor ligation necessary**
- **Amplify longer genes and rare transcripts**

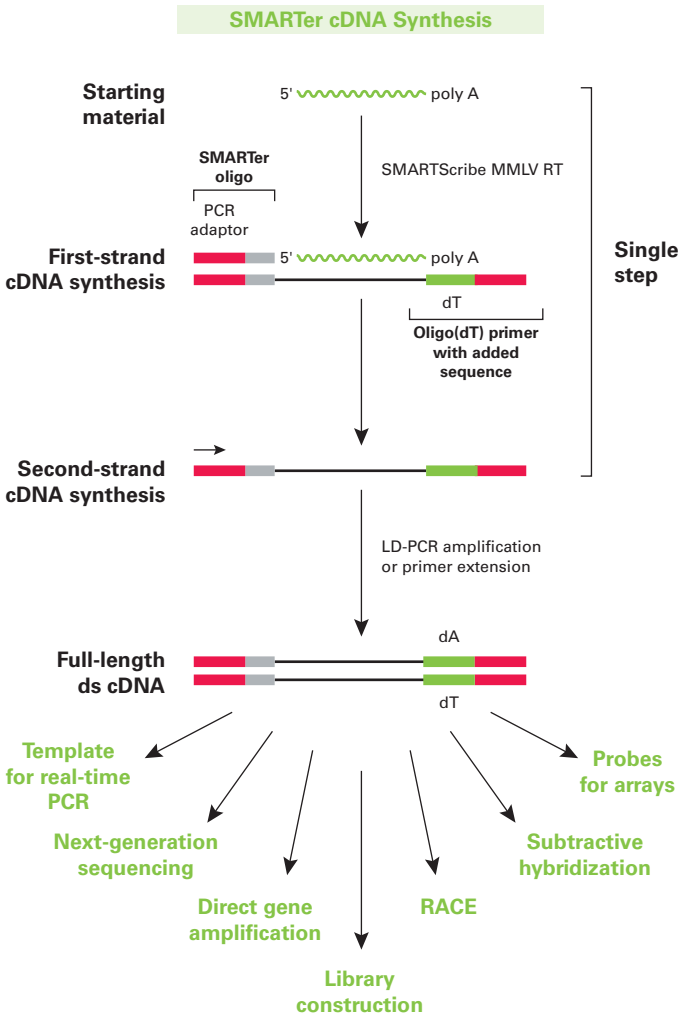
SMART™ Technology Just Got SMARTer

Clontech revolutionized cDNA synthesis with the invention of SMART (**S**witching **M**echanism **A**t the 5' end of **R**NA **T**ranscript) technology. This unique technology utilizes the intrinsic terminal transferase and template-switching activity of Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV RT) to accurately synthesize full-length cDNA in a single reverse transcription reaction (1), while incorporating adaptors on both ends of the cDNA. Incorporation of these universal primer binding sites in a single-step during first-strand cDNA synthesis eliminates the need for tedious second-strand synthesis and adaptor ligation, and facilitates a number of downstream applications, including RACE (**R**apid **A**mplification of **c**DNA **E**nds), subtractive hybridization, and library construction. This simple and highly efficient cDNA synthesis method ensures higher specificity in amplifying your target cDNA, compared to conventional methods.

Clontech has recently developed a new batch of kits featuring SMART technology with improved components—the SMARTer Kits for all your cDNA synthesis applications. These new SMARTer Kits include a modified **SMARTer II A Oligonucleotide** and **SMARTScribe™ Reverse Transcriptase.** The template-switching ability of SMARTScribe Reverse Transcriptase is enhanced when combined with the new SMARTer oligonucleotide; together these new components increase the likelihood of cloning your entire gene sequence, and result in high-quality, full-length cDNA, regardless of template size or abundance (2).

Get High-Quality cDNA from Less RNA

SMARTer cDNA Synthesis Kits are especially useful for researchers who have limited starting material, such as RNA derived from laser-capture microscopy samples, cells sorted by flow cytometry, or other extremely small samples. The **SMARTer PCR cDNA Synthesis Kit** allows first-strand cDNA synthesis from only 2 ng of total RNA (Figure 1, Panel A), much less starting material than is required



SMARTer cDNA synthesis occurs in a single-step reverse transcription reaction. Following amplification, SMARTer cDNA can be used for a variety of downstream applications.

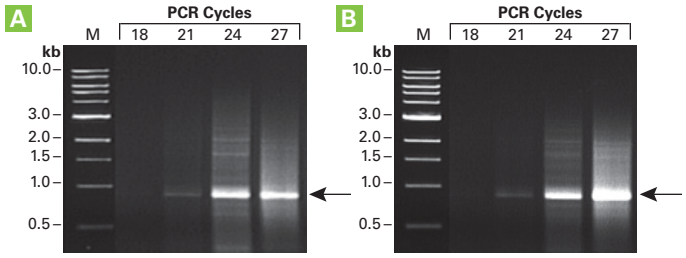


Figure 1. Typical gel profile of ds cDNA synthesized with the SMARTer PCR cDNA Synthesis Kit (Panel A) and the SMARTer Pico PCR cDNA Synthesis Kit (Panel B), using the Control Human Placental Total RNA as a template. 2 ng (**Panel A**) or 1 ng (**Panel B**) of the Control Human Placental Total RNA was subjected to first-strand cDNA synthesis and purification, followed by PCR amplification as described in the user manual for each kit. 5 µl of each PCR product was electrophoresed on a 1.2% agarose/EtBr gel in 1X TAE buffer following the indicated number of PCR cycles. Lanes M: 1 kb DNA ladder size markers. The arrows indicate the strong band at 900 bp typically seen for human placental total RNA.

for the original SMART PCR cDNA Synthesis Kit (Cat. No. 634902). Since extremely dilute RNA cannot be used in regular cDNA synthesis, we designed the new **SMARTer Pico PCR cDNA Synthesis Kit** to synthesize high-quality cDNA from even less starting material—as little as 1 ng of total RNA at a concentration as low as 20 pg/µl (Figure 1, Panel B). The SMARTer Pico Kit is the new and improved version of our original Super SMART PCR cDNA Synthesis Kit (Cat. No. 635000). The SMARTer Pico cDNA synthesis protocol makes it possible to use the entire volume of purified single-stranded cDNA for amplification, via increased reaction volumes and an additional column purification step. Both the SMARTer and SMARTer Pico protocols produce ds cDNA yields ranging from 1–2 µg.

Amplify Longer Transcripts

Clontech's new **SMARTer RACE cDNA Amplification Kit,** an improved version of our original SMART RACE cDNA Amplification Kit (Cat. No. 634914), allows you to identify the complete sequence of your RNA transcript from a small region of known sequence within the transcript all the way to the 5'- or 3'-end of the RNA, starting with as little as 2 ng of total RNA.

Following reverse transcription, SMARTer cDNA can be used directly in 5'- and 3'-RACE PCR reactions, without an additional adaptor ligation step. A side-by-side test of the new SMARTer oligo and the SMART oligo included in the original kit revealed an increased overall yield of 5'-RACE PCR products with the SMARTer oligo (Figure 2). The SMARTer RACE Kit facilitates RACE PCR via the included **Universal Primer Mix,** which is also sold separately. Additionally, the kit includes **random primers** for researchers whose RNA lacks a poly(A) tail.

References

1. Chenchik, A. *et al.* (1998) In *Gene Cloning and Analysis by RT-PCR.* Eds Siebert, P. & Larrick, J. (*BioTechniques* Books, MA) Ch 22.
2. Be SMART™ About First-Strand cDNA Synthesis (January 2009) *Clontechniques* **XXIV**(1):15–17.

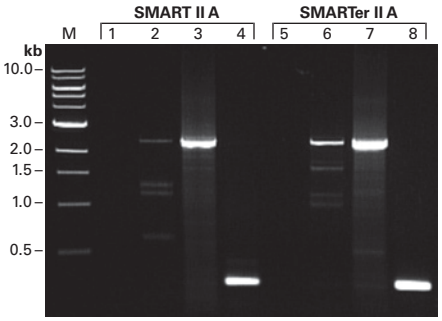


Figure 2. The SMARTer II A Oligo increases the overall yield of the 5'-RACE PCR products. Note the difference in intensity between the 2.6 kb 5'-RACE product in Lane 2 (produced by the SMART II A oligo) and that in Lane 6 (produced by the SMARTer II A oligo); both bands resulted from the amplification of only 2 ng of human placental total RNA template. In the assay shown, first-strand cDNA synthesis and 5'-RACE PCR amplification were performed as described in the user manual (PT4096-1). Lanes 1 & 5: No RNA 5'-RACE control. Lanes 2 & 6: Transferrin Receptor (TFR) 5'-RACE starting from 2 ng total RNA. Lanes 3 & 7: TFR 5'-RACE starting from 50 ng total RNA. Lanes 4 & 8: 5'-cDNA internal control using 2 ng total RNA as template. Lane M: 1 kb DNA Ladder.

Ordering Information

Product	Size	Cat. No.	
SMARTer™ PCR cDNA Synthesis Kit	10 rxns 20 rxns	634925 634926	NEW!
SMARTer™ Pico PCR cDNA Synthesis Kit	10 rxns	634928	NEW!
SMARTer™ RACE cDNA Amplification Kit	10 rxns 20 rxns	634923 634924	NEW!
Universal Primer Mix	100 rxns	634922	NEW!

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Easily Identify and Characterize Protein-DNA Interactions with Our New Yeast One-Hybrid System

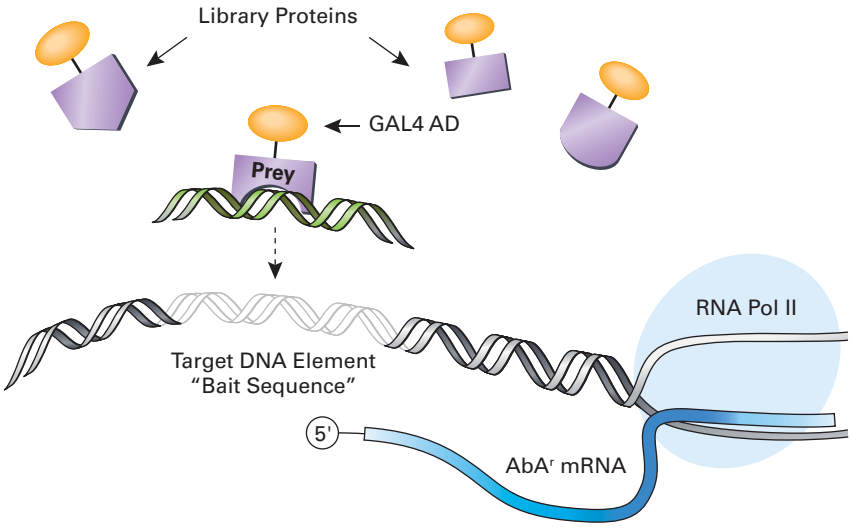
- **Highest performing yeast one-hybrid system**
- **Aureobasidin A selection eliminates background**
- **Complete system for easy construction and screening of cDNA libraries directly in yeast**

Clontech's **Matchmaker™ Gold Yeast One-Hybrid Library Screening System** provides a simple and efficient method for identifying and characterizing novel protein-DNA interactions. The system uses SMART™ cDNA synthesis technology, which allows cDNA libraries to be created from any tissue source, starting with as little as 100 ng of total RNA. It also employs **Aureobasidin A** (AbA; 1) selection, which provides the most stringent yeast one-hybrid (Y1H) screening strategy available.

The System

In the Matchmaker Gold Yeast One-Hybrid Library Screening System, 1–3 copies of your target DNA sequence (i.e., the bait) are cloned into the reporter vector pAbAi. The resulting pBait-AbAi construct is then integrated into the genome of the Y1HGold yeast strain by homologous recombination to generate a bait-specific reporter strain.

A cDNA library expressing potential DNA-binding proteins (i.e., prey) as fusions to the GAL4 transcription activation domain (AD) is constructed directly in the pBait-AbAi reporter strain. When a prey protein binds to the DNA target sequence (see figure below), transcription of the Aureobasidin A resistance gene (AbA^r) is activated, allowing the cell to grow on medium containing the antibiotic Aureobasidin A (AbA). In library screens, the plasmids encoding the library-derived prey proteins can be rescued from the surviving yeast clones and subjected to further analysis.



Screening for protein-DNA interactions with the Matchmaker Gold Yeast One-Hybrid System.

SMART Technology

The cDNA inserts for the prey library are created by SMART cDNA synthesis, which results in the incorporation of known primer sequences at both ends of the cDNA. Consequently, SMART-generated cDNA:

- *is available for amplification by PCR*—allowing the construction of libraries from nanogram amounts of starting RNA.
- *is flanked by sequences that are homologous to the cloning site of the linearized library vector, pGADT7-Rec*—allowing homologous recombination between the cDNA and the pGADT7-Rec vector upon transformation into the bait-specific reporter strain (Figure 1).

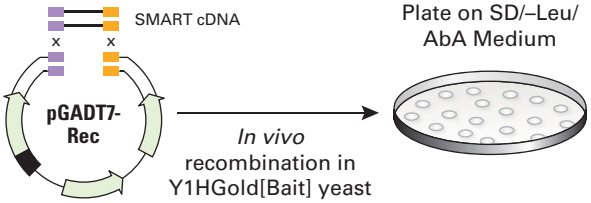


Figure 1. Use SMART technology and yeast biology to construct and screen your library. Your library is simultaneously constructed and screened directly in yeast. First, SMART cDNA synthesis technology is used to create a pool of cDNA that is flanked by sequences homologous to the ends of the linearized pGADT7-Rec vector. Next, the newly created Y1HGold-Bait reporter strain is transformed with the cDNA pool and pGADT7-Rec, which undergo homologous recombination within yeast. The yeast cells are then plated on SD/–Leu/+AbA to select for colonies that contain an active reporter (i.e., positive Y1H interactions).

Get Screening Results Fast!

With the Matchmaker Gold Yeast One-Hybrid Library Screening System, one-hybrid screening can be accomplished quickly and easily with the following steps:

Step 1. Create a bait construct by cloning 1–3 copies of the target DNA-binding sequence into pAbAi.

Step 2. Create a bait-specific reporter strain by transforming and integrating the linearized pBait-AbAi construct into the Y1HGold yeast strain and selecting on SD/–Ura medium, available in our **Yeast Media Set 1 Plus**.

Step 3. Confirm the integration of the bait sequence by colony PCR using **Matchmaker Insert Check PCR Mix 1**.

Step 4. Use SMART technology to synthesize cDNA that is flanked by sequences that are homologous to the ends of the linearized pGADT7-Rec vector.

Step 5. *Create and screen your Y1H library in a single step:* Cotransform your bait-specific Y1HGold reporter strain with the SMART-generated cDNA and pGADT7-Rec vector, and plate on SD/–Leu/+AbA.

Step 6. Harvest the resulting colonies, which contain putative DNA-binding proteins, and analyze further (e.g., with the **Matchmaker Insert Check PCR Mix 2** and the **Easy Yeast Plasmid Isolation Kit**).

The Matchmaker Gold Yeast One-Hybrid Library Screening System is the most convenient and advanced Y1H screening tool available, allowing library screens to be accomplished in less time and with greater confidence than ever before.

Aureobasidin A Selection Eliminates Background

Matchmaker Gold Systems are unique because they use the AbA^r gene as a novel reporter that confers resistance to AbA, a potent antifungal agent that is toxic to *S. cerevisiae*. Selecting for resistance to this highly stable depsipeptide makes Y1H library screening very straightforward (Figure 1), as AbA effectively kills yeast cells that are not expressing the AbA^r reporter. Aureobasidin A and all of the required media are supplied in our **Yeast Media Set 1 Plus.**

Reference

1. Takesako, K. *et al.* (1991) *J. Antibiot.* **44**(9):919–924.

Ordering Information

Product	Size	Cat. No.	
Matchmaker Gold Yeast One-Hybrid Library Screening System	5 rxns	630491	NEW!
Yeast Media Set 1 Plus	each	630493	NEW!
Matchmaker Insert Check PCR Mix 1	100 rxns	630496	NEW!
Matchmaker Insert Check PCR Mix 2	100 rxns	630497	NEW!
Easy Yeast Plasmid Isolation Kit	50 preps	630467	

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Proteome at Your Fingertips

Applications for the Ab Microarray 500

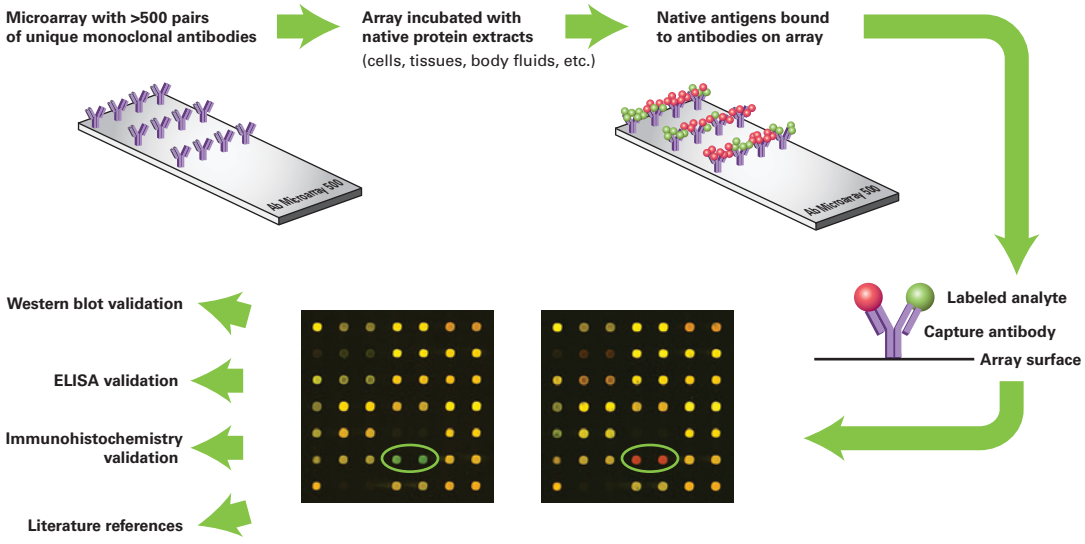
- **Applications—biomarker discovery, cancer research, profiling disease states & more**
- **Rapid & flexible—Screen >500 antibodies in 1 day; analyze data in 1 hour**
- **Highly reliable—≥80% correlation with Western blot analysis**
- **Validated technology—over 40 papers to date**

Clontech's **Ab Microarray 500** is a robust, inexpensive tool for high-throughput analysis of proteomic profiles. This array can be used as a screening tool for biomarker discovery and target identification—as well as for rapid expression profiling of proteins in a variety of samples, including serum, tissues, cell lines, and cancer vs. normal or treated vs. untreated samples. For example, this technology has been used to identify overexpressed proteins in mantle cell lymphoma (1), yielding results that were validated using Western blot and immunohistochemistry.

The Technology

The Ab Microarray 500 is designed to perform rapid, reliable characterization of changes in protein expression between two samples. It allows scientists to focus their research on a smaller number of proteins that have biological relevance—by screening >500 proteins in one day. This array, which contains specific monoclonal antibodies, is incubated with Cy3™/Cy5™-labeled serum, tissue, or cell extract samples—in order to profile disease states, identify tumor-associated antigens, perform time course studies, discover biomarkers, and more. The antibodies on the array recognize human, mouse, and rat proteins.

High data quality is achieved by using a Cy3/Cy5 dye swap detection technology together with an internally normalized ratio (2). This technology also has a built-in concentration step that allows for the detection of low-abundance proteins by accumulating antigens onto the antibody during the incubation step—thus increasing the signal. A one-hour data analysis procedure yields highly sensitive, reproducible, and reliable data that are biologically relevant. These data have been validated using Western blotting, ELISA, immunohistochemistry, and literature references (over 40 publications to date).



Overview of the Ab Microarray 500 Protocol.

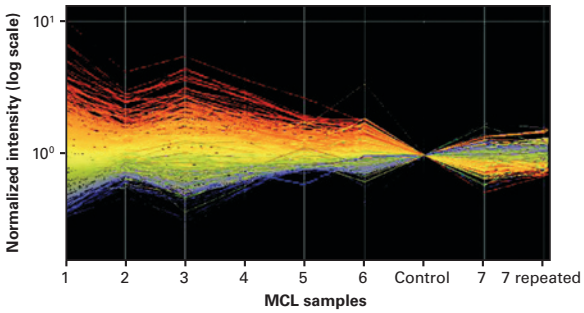


Figure 1. Expression levels of the 13 overexpressed proteins in the MCL samples as compared to the control. The data points are colored in red/orange, indicating overexpression. The samples are numbered 1 to 7. MCL7 showed a reversed expression pattern compared to the other 6 MCL samples. MCL7 was tested twice and demonstrated a consistent expression pattern. Data were analyzed using GeneSpring™ software (Agilent Technologies, Inc.)

Ab Microarray 500 Analysis of Mantle Cell Lymphoma

The Ab Microarray 500 was used in the proteomic analysis of mantle cell lymphoma (MCL; 1). Seven samples were analyzed and compared to a control sample. Six of the samples showed 13 proteins that were overexpressed when compared to the control, and one sample displayed an inverted expression profile (Figure 1). The latter sample was reanalyzed and the same results were observed.

Data Reproducibility

Six different histologically-confirmed MCL samples displayed the same protein expression profile, demonstrating the reliability of the array in providing consistent, accurate results. The reproducibility of the array data was also confirmed by the MCL7 sample that was analyzed twice (in 2 different experiments) and yielded the same result.

Validation of Results

The Ab Microarray 500 data from the mantle cell lymphoma analysis (Figure 1) was validated by Western blot (1; data not shown) and immunohistochemistry (Figure 2).

False Positives

Western blot analysis (1; data not shown) and immunohistochemistry (Figure 2) were used to confirm array data. Western blotting results were consistent with array results for 7 of the proteins identified, but not for 2 of them. One of the 2 proteins (Hsp90) also failed to show overexpression when analyzed by immunohistochemistry (Figure 2, Panel A).

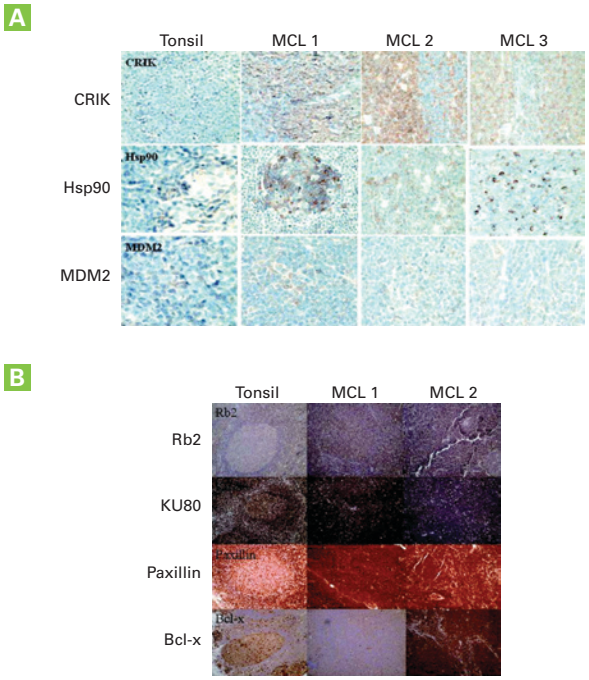


Figure 2. Immunohistochemistry was used to analyze false positive and false negative results. Paraffin-embedded tissue biopsies available from the same patients were analyzed using antibodies present on the array in order to test for false positives (**Panel A**) and false negatives (**Panel B**). The **Panel A** results are consistent with the antibody array in detecting increased levels of CRIK and MDM2, and the Western blot in failing to demonstrate elevated Hsp90 levels. The **Panel B** results did not reveal any false negatives for the proteins analyzed.

False Negatives

Four proteins that did not show changes on the array were analyzed by immunohistochemistry (Figure 2, Panel B). None of the 4 proteins revealed any changes when analyzed by this method, proving that the array does not yield false negatives.

Conclusion

Comparing relative expression levels with the Ab Microarray 500 revealed the existence of 3 novel proteins associated with mantle cell lymphoma. The results demonstrate the capacity of the Ab Microarray 500 for identifying novel proteins as well as the importance of confirming antibody array data using an independent method.

References

1. Ghobrial, I. M. *et al.* (2005) *Blood* **105**(9):3722–3730.
2. Andersson, O. *et al.* (2005) *J. Proteome Res.* **4**(3):758–767.

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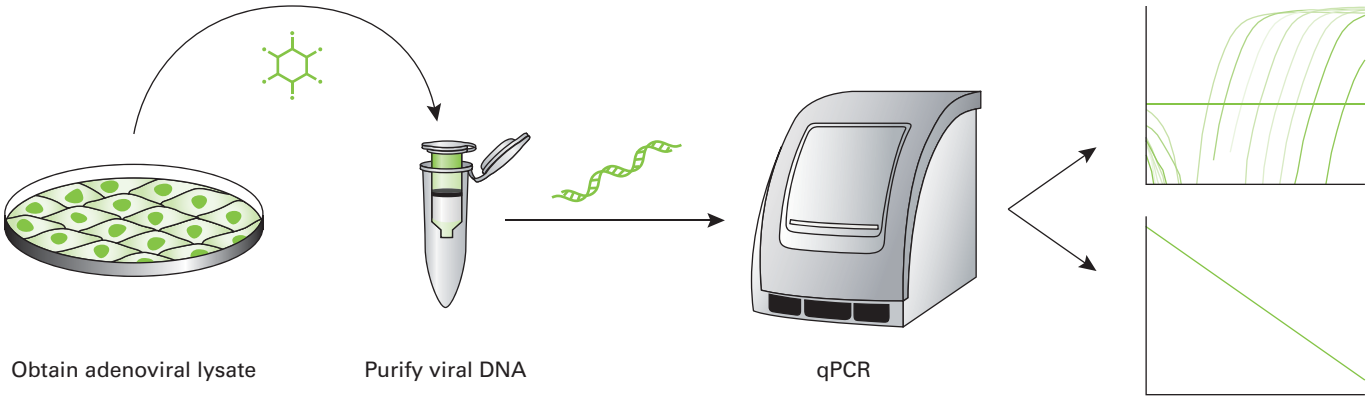
Product	**Size**	**Cat. No.**
Ab Microarray 500 Slides	2 arrays	631790
Ab Microarray Express Buffer Kit	each	631795

Obtain Adenoviral Titers in Less than 4 Hours

Rapidly determine adenoviral titers with our new Adeno-X™ qPCR Titration Kit

- **Harvest, titrate, and infect in a single day**
- **Measure titers from crude lysate or purified viral preps**

The **Adeno-X qPCR Titration Kit** provides an extremely fast, simple, and accurate method for titrating adenoviral stocks from all Ad5-based adenoviral vectors. The kit delivers results in just 4 hours, a vast improvement over standard titration methods (such as the plaque assay), which require up to 10 days to complete. Because qPCR titration is so fast, target cells can be infected with accurately titrated virus on the same day the virus is harvested.



Flowchart of the procedures used for titrating adenoviral DNA with the Adeno-X qPCR Titration Kit.

Table I: Comparison of Adeno-X qPCR Titration to Other Titration Methods

		Titration Method			Adeno-X qPCR Titration Ratios	
Sample Type	**Virus**	**qPCR[a]** (copies/ml)	**Fluor[b]** (IFU/ml)	**X-Gal** (IFU/ml)	**qPCR/Fluor** (copies/IFU)	**qPCR/X-Gal** (copies/IFU)
Purified	AdAcGFP1	5.62×10^9	3.03×10^9	N/A	2	N/A
Crude	AdAcGFP1					
	Prep A	1.44×10^{10}	1.90×10^9	N/A	8	N/A
	Prep B	1.46×10^{10}	2.26×10^9	N/A	6	N/A
	Prep C	1.38×10^{10}	2.73×10^9	N/A	5	N/A
Purified	AdLacZ	1.01×10^{10}	N/A	1.54×10^9	N/A	7
Crude	AdLacZ					
	Prep A	1.33×10^{10}	N/A	2.67×10^9	N/A	5
	Prep B	1.24×10^{10}	N/A	2.67×10^9	N/A	5
	Prep C	1.21×10^{10}	N/A	3.15×10^9	N/A	4

a Adenoviral copy numbers were determined using the Adeno-X qPCR Titration Kit (Cat. No. 632252).
b To determine fluorescence-based infectivity titers, adenoviral stocks were serially diluted (tenfold) and applied to HEK 293 cells. After 48 hr, fluorescent cells were scored with a fluorescence microscope.

The qPCR Titration Method

The protocol combines qPCR with SYBR® Green chemistry, allowing you to determine the viral genome copy number in adenoviral preparations (i.e., crude lysates or purified stocks) from a calibrated DNA standard curve (Figure 1). The procedure is simple: viral DNA and control DNA (provided) are serially diluted and subjected to qPCR. The DNA copy number of each viral sample is then determined by comparing its C_t value to a standard curve generated by plotting the C_t values of the diluted control samples against their respective copy numbers (Figure 1). Titration assays demonstrating the consistency of this approach when used with either crude lysate or purified virus are shown in Figure 2.

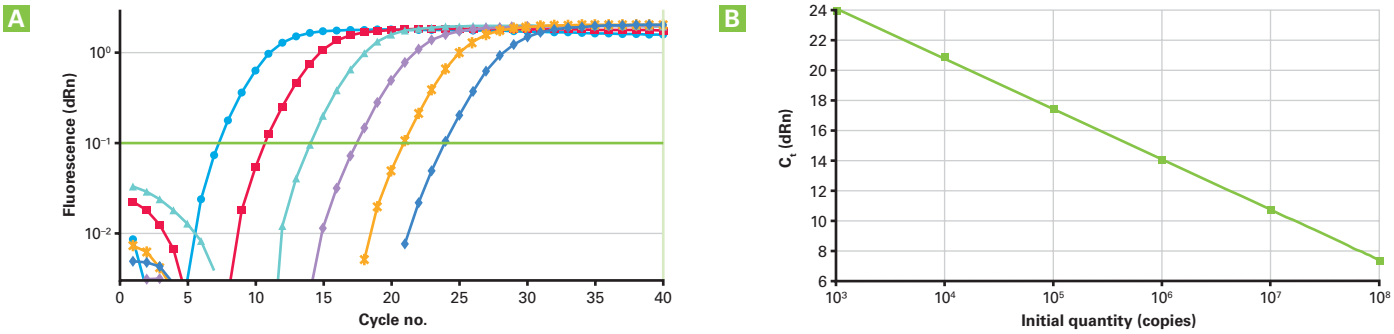


Figure 1. The Adeno-X qPCR titration method exhibits a wide dynamic range. Adeno-X DNA Control Template was serially diluted to 10^8–10^3 copies per sample and analyzed with the Adeno-X qPCR Titration Kit. The amplification plots (**Panel A**) show a dynamic range of at least six orders of magnitude (each dilution is represented by a different colored plot). The standard curve (**Panel B**) demonstrates a strong linear correlation between the C_t and the DNA copy number (log scale), with R^2 = 1.000 and a PCR efficiency of 96.2%.

Once the genome copy number of your viral stock is determined, it can be correlated with the number of viral infectious units (IFU; determined independently) to establish a copy number/IFU relationship (Table I). Determination of the copy number/IFU relationship for a given prep allows you to normalize the amount of the prep used in each experiment, for consistent interassay results.

Our method makes it possible to infect cells at a known multiplicity of infection (MOI), allowing you to produce results that are precise, consistent, and interpretable. The Adeno-X qPCR Titration Kit includes sufficient material for 200 qPCR reactions and allows multiple virus preparations to be titrated simultaneously.

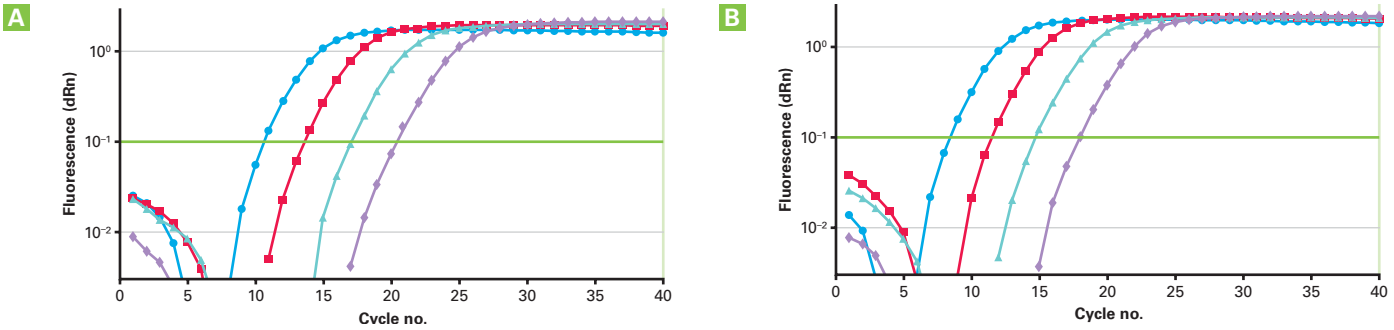


Figure 2. Titrate your adenovirus from crude lysate or purified viral particles. The Adeno-X qPCR Titration kit was used to titrate capsid-free viral DNA obtained from both crude lysate (**Panel A**) and purified viral particles (**Panel B**). In both cases, the capsid-free DNA was obtained with a virus purification kit (provided with the Adeno-X qPCR Titration Kit) and serially diluted (10X) prior to qPCR (each dilution is represented by a different colored plot). The titration procedure worked equally well regardless of the purity of the viral particles.

Ordering Information			
Product	**Size**	**Cat. No.**	
Adeno-X qPCR Titration Kit	200 rxns	632252	**NEW!**

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Live Cell Reporters, Now with Lentiviral Delivery

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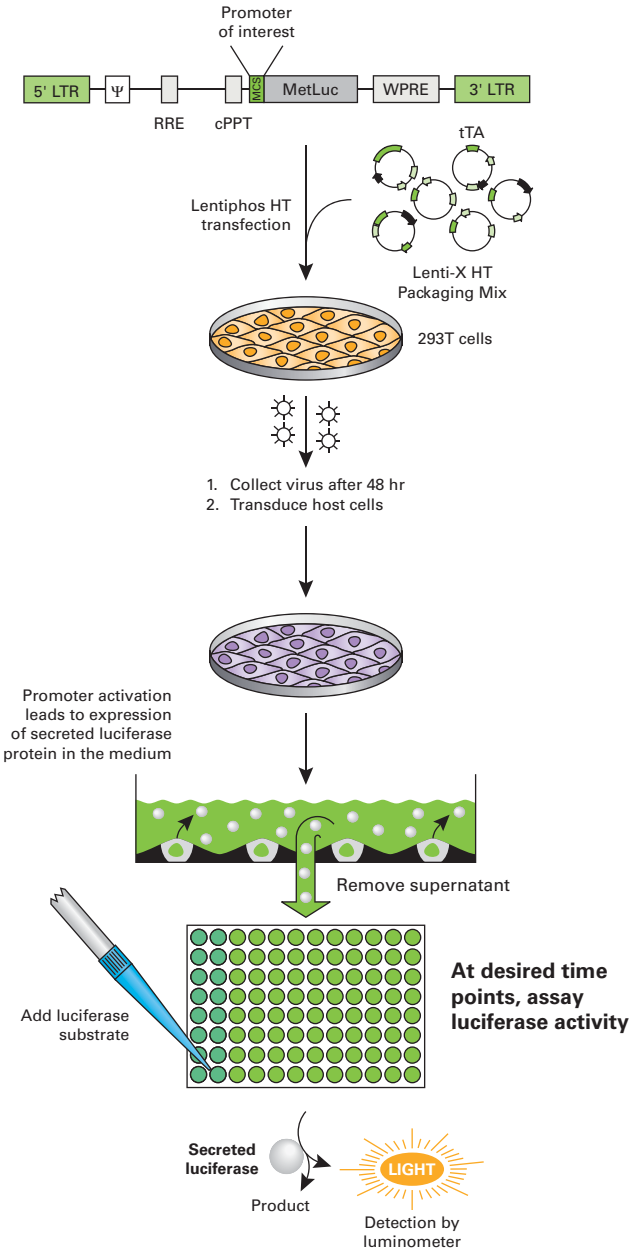
- **Choose chemiluminescent or fluorescent reporters**
- **Measure promoter activity on demand**
- **Get more data points per experiment**

Clontech's **Lenti-X™ Reporter Systems** allow you to study your promoter of interest with a chemiluminescent or fluorescent on-demand reporter in virtually any cell type, including primary cultures, dividing and nondividing cells, stem cells, terminally differentiated cells, and neuronal cells. These are complete systems, which include our advanced 4th-generation packaging systems and lentivirus transfection system, plus your vector of choice (1; Figure 1). They deliver excellent signal-to-noise ratios by excluding reporter molecules that are expressed prior to the experiment (Figure 2) and allow you to monitor promoter activity at any time point and for any length of time that you choose. Because there is no cell lysis, you can observe multiple promoter activation cycles using the same cells.

Table I: How Do I Choose My Reporter System?

	Lenti-X Ready-To-Glow Secreted Luciferase Reporter System	Lenti-X DD Fluorescent Protein[1] Reporter Systems
Chemiluminescent detection (plate reader)	✓	
Fluorescent detection (flow cytometry, fluorescence microscopy)		✓
Automatable	✓	✓
License required for For-Profit organizations		✓

1 AmCyan1, ZsGreen1, or tdTomato



Flowchart of the Lenti-X Ready-To-Glow Secreted Luciferase Reporter System protocol.

Don't Miss Transient Activity

The **Lenti-X Ready-To-Glow™ Secreted Luciferase Reporter System** is based on a secreted reporter, *Metridia* luciferase, which exhibits superior performance compared to traditional cytosolic luciferase reporters. Secreted luciferases are not degraded like cytosolic luciferase reporters, so you can monitor intermittent short bursts of promoter activity that you might have missed previously. In addition, *Metridia* luciferase has higher sensitivity than *Renilla* or firefly luciferases (2). To remove background, just "wash out" any reporter molecules synthesized prior to your experiment with a simple media change. Then activate the promoter and monitor its activity by sampling the accumulated stable reporter from the media.

Measure Activity on Demand

The **Lenti-X DD Fluorescent Protein Reporter Systems** combine a bright fluorescent protein reporter for high signal intensity with ProteoTuner™ technology to eliminate background (3). Ligand-dependent, on-demand stabilization of the fluorescent reporter allows you to start monitoring promoter activation whenever you choose.

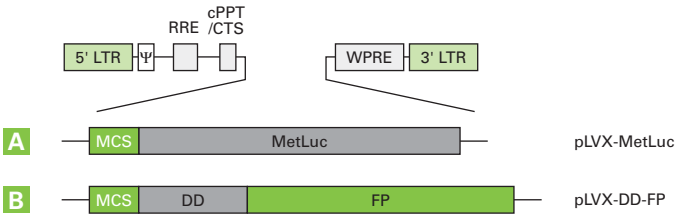


Figure 1. Lenti-X chemiluminescent (Panel A) and fluorescent (Panel B) reporter vectors. Lenti-X vectors contain sequence elements that facilitate lentiviral packaging and/or boost expression of your reporter, including the LTRs, packaging signal (Ψ), Rev response element (RRE), and central polypurine tract/central termination sequence (cPPT/CTS) from HIV-1; and the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). MetLuc = *Metridia* luciferase. DD = ligand-dependent destabilization domain. FP = fluorescent protein (AmCyan1, ZsGreen1, or tdTomato).

Save Time and Money Using Live Cell Reporters

These systems are ideal for your studies with limited numbers of cells, e.g., stem cells, or to study multiple promoter activation cycles and/or time points, in order to produce many sets of data over time.

References

1. High-Efficiency Lentiviral Packaging (October 2007) *Clontechniques* **XXII**(4): 1–2.
2. Ready-To-Glow™ Secreted Luciferase System (July 2006) *Clontechniques* **XXI**(2):12–13.
3. The Next Generation of Promoter Reporters (January 2009) *Clontechniques* **XXIV**(1)22–23.

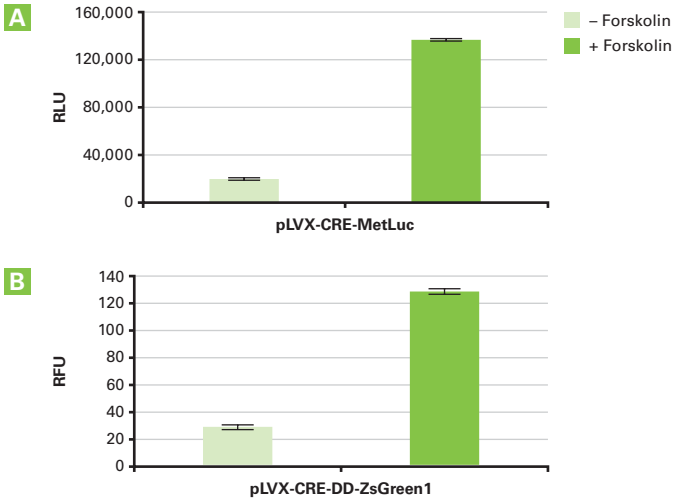


Figure 2. Lenti-X Reporter Systems provide low background and high signal intensity. HEK 293 cells were transduced with pLVX-CRE-MetLuc Reporter Vector (**Panel A**) or pLVX-CRE-DD-ZsGreen1 Reporter Vector (**Panel B**), treated with forskolin, and assayed according to the respective protocols. RLU = relative light units. RFU = relative fluorescence units.

Ordering Information

Product	Size	Cat. No.	
Lenti-X Ready-To-Glow Secreted Luciferase Reporter System	each	631746	NEW!
Lenti-X DD Cyan Reporter System	each	631748	NEW!
Lenti-X DD Green Reporter System	each	631751	NEW!
Lenti-X DD Red Reporter System	each	631753	NEW!
Lenti-X 293T Cell Line	1 ml	632180	
Shield1*	60 µl 200 µl 500 µl	631037 631038 632189	

* The number of reactions depends on the concentration of Shield1 used. At the maximum suggested concentration (1,000 nM), 60 µl = 30-plus reactions and 200 µl = 1,000-plus reactions in a six-well plate.

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Concentrate Lentivirus… Effortlessly

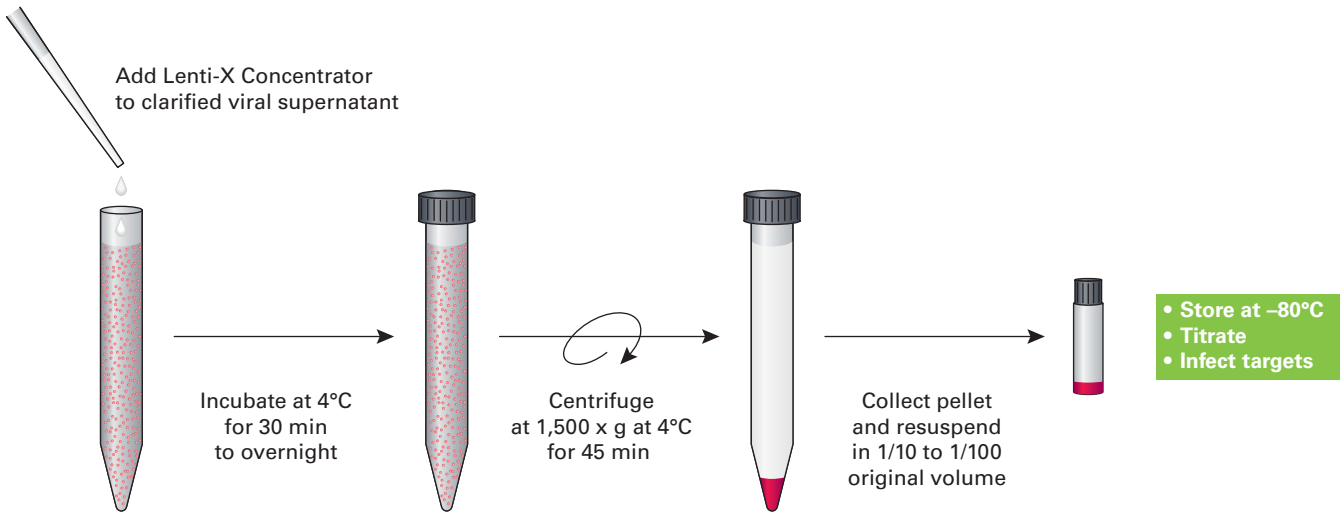
Increase your viral titer 100-fold with Lenti-X™ Concentrator—without ultracentrifugation

- **Simply mix and spin**
- **Hassle-free and easily scaled up for large volumes**
- **No ultracentrifugation required**

Need to concentrate your lentivirus preps, but don't want the hassle of ultracentrifugation? Use **Lenti-X Concentrator** to increase your available titer up to 100-fold and infect your target cells at higher MOIs and in reduced volumes, without making more virus.

Simple Protocol: Mix, Wait, Spin

Lenti-X Concentrator provides a fast, simple, and highly efficient method for concentrating any lentiviral stock. In the simple protocol, you just mix your lentiviral supernatant with the Lenti-X Concentrator reagent, incubate for a short period, and spin the mixture in a standard centrifuge (see below). You'll increase your vector titer by up to 100-fold in ~1 hr, and obtain excellent recoveries—with no ultracentrifugation. Lenti-X Concentrator works for all lentiviral supernatants, including those made from any of Clontech's Lenti-X Systems, and the procedure can be scaled up or down to best suit your needs.



The Lenti-X Concentrator protocol. Add Lenti-X Concentrator reagent to clarified viral supernatant, incubate for 30 min to overnight at 4°C, and spin. That's it.

Increase Titers by 100-fold

Using the Lenti-X Concentrator protocol, we were able to increase the titer of a lentiviral supernatant from 10^7 to 10^9 IFU/ml, and recover 90% of the virus in 1/100 of the original volume (Figure 1). You can achieve similar results starting with any volume of supernatant.

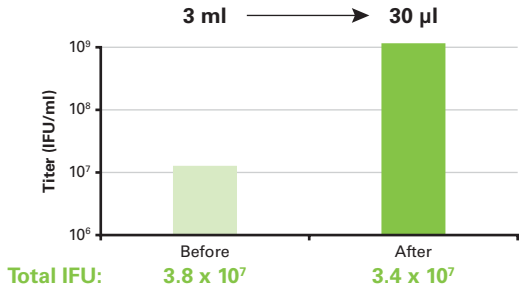


Figure 1. Efficient concentration with minimal loss. Lentiviral supernatant from a pLVX-ZsGreen1 vector was concentrated from 3 ml down to 30 µl using the Lenti-X Concentrator reagent, which reflected a 100-fold increase in viral titer. Measuring the total amount of virus contained in each sample indicated that the resuspended pellet captured 90% of the virus present in the original sample. Samples were titrated using HT1080 cells and analyzed by flow cytometry 48 hr post-transduction.

Far Simpler Than Ultracentrifugation

In a side-by-side comparison between Lenti-X Concentrator and ultracentrifugation, the advantages of Lenti-X are clearly evident (Table I). Lenti-X Concentrator is more flexible, faster, easier, and just as efficient as ultracentrifugation.

Table I: Lenti-X Concentrator vs. Ultracentrifugation

Feature	Lenti-X Concentrator	Ultracentrifugation
Easily Scalable	Yes	No
Specialized Equipment	No	Yes
Time Required	~1 hr	4 hr to overnight
Ease-of-Use	++++	+
Yield	>90%	>90%

Scalable for Supernatants of Any Volume, Any Titer

The Lenti-X Concentrator reagent is itself a 4X concentrate, so it can be added to any volume of supernatant containing any amount of virus or any starting titer. To illustrate, we diluted a sample of supernatant to 250 ml, then concentrated the virus and resuspended it in 2.5 ml, recovering >95% of the original virus (Figure 2). We also concentrated virus from serially diluted samples of lentiviral supernatant which were reduced to 1/100 of their original volumes using the Lenti-X Concentrator protocol (Figure 3). Regardless of the starting titer or volume, virtually all of the virus is recovered in the Lenti-X Concentrator pellets.

Whether you need to reduce the volume of your viral supernatant, or increase its titer, Lenti-X Concentrator produces the results you need—quickly and simply, without the time-consuming hassles of ultracentrifugation.

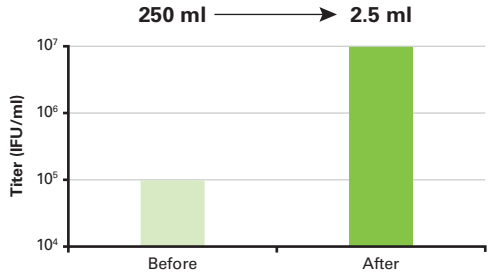


Figure 2. Concentrate virus from large volumes. Lentiviral supernatant was diluted into 250 ml and then concentrated down to 2.5 ml using Lenti-X Concentrator. Titrations were performed using HT1080 cells and flow cytometry 48 hr post-transduction.

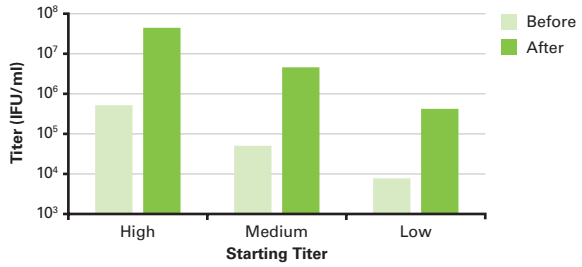


Figure 3. Concentrate virus from any starting titer. Tenfold serial dilutions of a high-titer lentiviral supernatant (high, medium, and low) were concentrated from a volume of 10 ml down to 100 µl using the Lenti-X Concentrator Reagent. Titrations were performed using HT1080 cells and flow cytometry 48 hr post-transduction.

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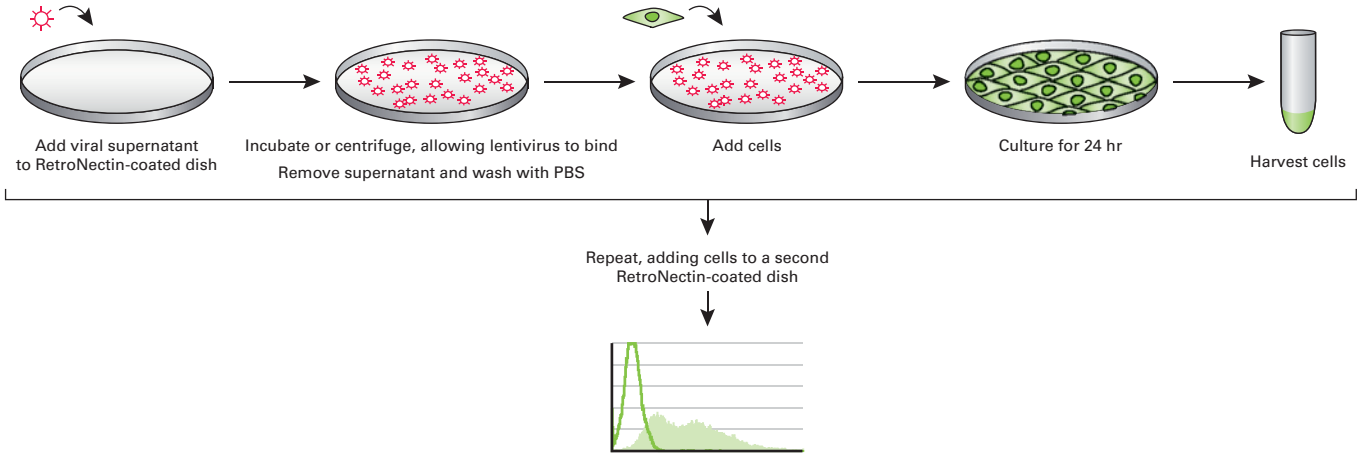
Ordering Information

Product	Size	Cat. No.	
Lenti-X Concentrator	100 ml	631231	NEW!
	500 ml	631232	

Excellent Infection Rates with Lenti-X™ Systems and the RetroNectin® Reagent

A simple protocol for difficult-to-transduce cells

Why a superinfection? We have used our Lenti-X viral systems together with RetroNectin-coated dishes from Takara Bio to successfully transduce Jurkat T cells, which can transduce inefficiently. This double infection protocol increases the infection efficiency for Jurkat cells from <10% to >90% (1). To read the specifics of our experiment, please visit us on the web at **www.clontech.com/jurkat**



Flowchart protocol for infecting Jurkat cells with Lenti-X vectors, using RetroNectin plates. Using this protocol drastically increases transduction efficiency (1).

Reference

1. Improve Viral Transductions with RetroNectin Reagent (October 2008) *Clontechniques* **XXIII**(4):7–8.

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Ordering Information		
Product	**Size**	**Cat. No.**
Lenti-X Expression System	each	632164
Lenti-X HT Packaging System	20 rxns 40 rxns	632160 632161
Lenti-X HT Ecotropic Packaging System	20 rxns	632199
Lenti-X 293T Cell Line	1 ml	632180
RetroNectin Reagent	0.5 mg 5 x 0.5 mg	TAKT100A TAKT100B
RetroNectin Precoated Dishes	10 dishes	TAKT110A

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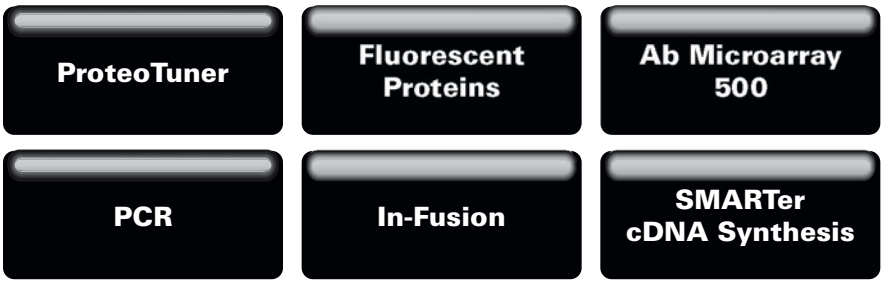

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